

EZ-Link Maleimide-PEG₂-Biotin

MAN0016363

Rev. B.0

Pub. Part No. 2160748

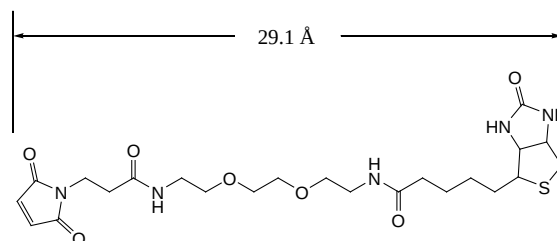
21901BID A39261

Number	Description
21901BID	EZ-Link Maleimide-PEG ₂ -Biotin, 50mg
A39261	EZ-Link Maleimide-PEG ₂ -Biotin, No-Weigh Format, 10 × 2mg vials

Formula: C₂₃H₃₅N₅O₇S

Molecular Weight: 525.62

Spacer Arm Length: 29.1Å

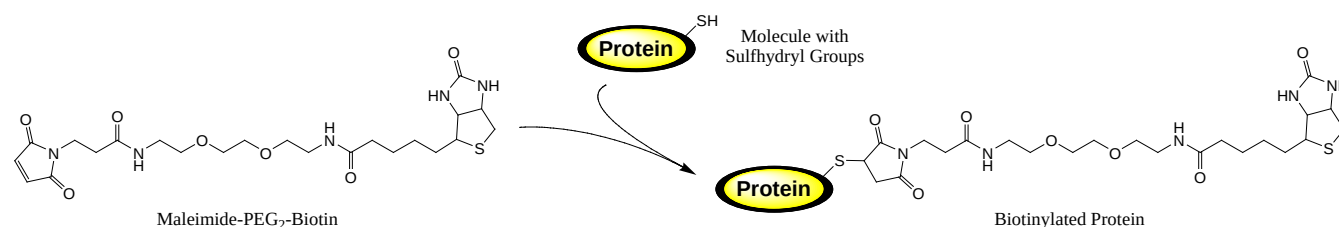
Maximum Solubility: ≥ 25mg/mL
in water or buffer**Storage:** Upon receipt store at 4°C. Product shipped at ambient temperature.**Note:** Product labels have been provided for your convenience. Please label the vials using one of the labels provided in the Al foil pouch to avoid any confusion as you work with this No-Weigh reagent.

Introduction

Thermo Scientific™ EZ-Link™ Maleimide-PEG₂-Biotin is a water-soluble sulfhydryl-reactive biotinylation reagent. The polyethylene glycol (PEG) spacer arm has a hydrophilic property that is transferred to the final biotin conjugate, helping to prevent aggregation of labeled antibodies stored in solution. Typical PEG reagents contain heterogeneous mixtures of different PEG chain lengths; however, Thermo Scientific™ Pierce™ PEG reagents are homogeneous compounds of defined molecular weight and spacer arm length, providing precision in optimizing modification applications.

Biotin is a small naturally occurring vitamin that binds with high affinity to avidin and streptavidin proteins. Biotinylated proteins typically retain biological activity because the biotin group is relatively small. An antibody conjugated with several biotin molecules can amplify signal, thereby increasing the sensitivity of many assays. The bond formation between biotin and avidin is rapid and, once formed, is unaffected by most extremes of pH, organic solvents and other denaturing agents. Labeled proteins can be purified using immobilized streptavidin, avidin or Thermo Scientific™ NeutrAvidin™ Affinity Resins (see Related Thermo Scientific Products) and detected in ELISA, dot blot or Western blot applications.

Maleimide-activated reagents are effective for protein modification of sulfhydryl groups. Maleimide groups react efficiently and specifically with free (reduced) sulfhydryls at pH 6.5-7.5 to form stable thioether bonds (Figure 1). Most proteins have cysteine residues whose side-chain sulfur atoms typically occur in pairs as disulfide bonds. Reduction of these disulfide bonds exposes the sulfhydryl group required as a target for biotinylation with maleimide-activated reagents. Alternatively, sulfhydryl groups can be added to molecules using various modification reagents (see subsequent Important Product Information Section). Maleimide-PEG₂-Biotin is readily soluble in water or organic solvents such as dimethylsulfoxide (DMSO), methylene chloride or dimethylformamide (DMF).

**Figure 1.** Reaction scheme for biotinylation of sulfhydryl molecules with Maleimide-PEG₂-Biotin.

Important Product Information

- EZ-Link Maleimide PEG₂-Biotin is moisture-sensitive. Store product in the original container at 4°C with desiccant. Equilibrate vial to room temperature before opening to avoid moisture condensation onto the product. Prepare reagent solution immediately before use. The maleimide moiety will hydrolyze and become non-reactive in water; therefore, aqueous stock solutions cannot be prepared for storage. Discard any unused reconstituted reagent.
- Molecules to be reacted with the maleimide moiety must have free (reduced) sulfhydryls. Reduce peptide disulfide bonds with Thermo Scientific™ Immobilized TCEP Disulfide Reducing Gel (Product No. 77712). Reduce disulfide bonds in high molecular weight proteins using 5mM TCEP (1:100 dilution of TCEP Solution, Product No. 77720) for 30 minutes at room temperature, followed by TCEP removal using a desalting column (e.g., Thermo Scientific™ Zeba™ Spin Desalting Columns). Proteins (e.g., antibodies) can be inactivated by complete reduction of their disulfide bonds. Selective reduction of hinge-region disulfide bonds in IgG can be accomplished with 2-Mercaptoethylamine•HCl (2-MEA, Product No. 20408). Sulfhydryls can be added to molecules using *N*-succinimidyl *S*-acetylthioacetate (SATA, Product No. 26102 or SAT(PEG)₄, Product No. 26099) or 2-iminothiolane•HCl (Traut's Reagent, Product No. 26101), which modify primary amines.
- Avoid extraneous sulfhydryl-containing components in the reaction buffers during conjugation (e.g., DTT), as they react with the maleimide portion of the reagent, inhibiting and reducing conjugation efficiency of the intended target.
- The maleimide group reacts predominantly with free sulfhydryls at pH 6.5-7.5, forming stable thioether bonds. At pH values > 7.5, reactivity toward primary amines and hydrolysis of the maleimide groups can occur. At pH 7, the maleimide group is ~1000 times more reactive toward a free sulfhydryl than to an amine.
- If desired, excess nonreacted EZ-Link Maleimide-PEG₂-Biotin can be removed by size exclusion using either desalting columns or dialysis units (see Related Thermo Scientific Products).

Additional Materials Required

- Phosphate-buffered saline (PBS) or other sulfhydryl-free buffer having pH 6.5-7.5 for use as reaction buffer (see Important Product Information and Related Thermo Scientific Products)
- Desalting columns or dialysis units for buffer exchange and removal of excess reagent following modification (e.g., Zeba™ Spin Desalting Columns or Thermo Scientific™ Slide-A-Lyzer™ Dialysis Units, see Related Thermo Scientific Products)

Procedure for Biotinylation of Proteins with Maleimide-PEG₂-Biotin

The optimal amount of Maleimide-PEG₂-Biotin to use for each reaction depends on a number of factors. By regulating the reagent-to-target molar ratio in the reaction, the extent of labeling can be controlled. As a starting point use a 5- to 20-fold molar excess of reagent for protein solutions >2mg/mL. When labeling more dilute solutions, a greater relative molar fold excess of reagent may be necessary to achieve the same results. Optimal molar ratios for small molecule modification may differ significantly. Example calculations for IgG modification (molecular weight 150,000) are provided for convenience.

A. Calculations

1. Calculate the quantity in millimoles of the reagent to add to the reaction for a 20-fold molar excess:

$$\text{mL protein} \times \frac{\text{mg protein}}{\text{mL protein}} \times \frac{\text{mmol protein}}{\text{mg protein}} \times \frac{20 \text{ mmol Biotin Reagent}}{\text{mmol protein}} = \text{mmol Biotin Reagent}$$

Note: the value 20 in this equation corresponds to the suggested reagent molar fold excess for a 2mg/mL protein sample.

2. Calculate microliters of 20mM Biotin Reagent Stock Solution (prepared in Step B.2) to add to the reaction:

$$\text{mmol Biotin Reagent} \times \frac{1,000,000 \mu\text{L}}{\text{L}} \times \frac{\text{L}}{20 \text{ mmol}} = \mu\text{L Biotin Reagent Stock Solution}$$

Example: For 1mL of a 2mg/mL IgG (150,000 MW) solution, ~13μL of 20mM Biotin Reagent will be added.

$$1 \text{ mL IgG} \times \frac{2 \text{ mg IgG}}{1 \text{ mL IgG}} \times \frac{1 \text{ mmol IgG}}{150,000 \text{ mg IgG}} \times \frac{20 \text{ mmol Biotin Reagent}}{1 \text{ mmol IgG}} = 0.000266 \text{ mmol Biotin Reagent}$$

$$0.000266 \text{ mmol Biotin Reagent} \times \frac{1,000,000 \mu\text{L}}{\text{L}} \times \frac{\text{L}}{20 \text{ mmol}} = 13.3 \mu\text{L of 20 mM Biotin Reagent Stock Solution}$$

B. Biotin Labeling Reaction

1. Dissolve protein to be modified in sulfhydryl-free buffer at pH 6.5-7.5, according to the calculations made in Section A.

Note: Protein already in sulfhydryl-free buffer at pH 6.5-7.5 may be used without buffer exchange or dilution.

2. Immediately before use, add 190μL of PBS to 2mg of EZ-Link Maleimide-PEG₂-Biotin to prepare a 20mM stock solution.

Note: If using the Thermo Scientific™ No-Weigh™ EZ-Link Maleimide-PEG₂-Biotin, unscrew the vial and add 190μL of PBS and mix by pipetting up and down. Alternatively, the vial can be vortexed for a few seconds to ensure a homogeneous solution. **The maximum useable volume of the vial is 800μL.** Store the unused vials in the Al foil pouch at 4°C.

Note: It is possible to make a stable concentrated (100-200mM) stock solution of Maleimide-PEG₂-Biotin by dissolving the reagent in pure, moisture-free (“dry”) DMSO (Product No. D12345) or DMF (Product No. 20673). With proper handling (i.e., complete exclusion of moisture), the stock may be stable for several months at -20°C.

3. Add the appropriate volume of the EZ-Link Maleimide-PEG₂-Biotin (see Calculations Section) to the protein solution and mix.
4. Incubate reaction on ice or room temperature for two hours to overnight.

Note: Except for possible degradation or microbial growth, there is no harm in reacting longer than the specified time.

5. Labeling is complete at this point and, although excess nonreacted and hydrolyzed Biotin Reagent remains in the solution, it is often possible to perform preliminary tests of the labeled protein. Once proper function and labeling has been confirmed, the labeled protein may be purified from nonreacted Maleimide-PEG₂-Biotin by desalting or dialysis.

Troubleshooting

Problem	Possible Cause	Solution
Protein is not biotinylated	There were no available free sulfhydryls	Reduce existing disulfide bonds to generate free sulfhydryls, or introduce sulfhydryls with Traut's Reagent, SATA or SAT(PEG) ₄
	Maleimide group was hydrolyzed and non-reactive	Do not store reagent in aqueous solutions or solvent that has absorbed water

Determination of Biotin Incorporation

Biotin incorporation can be estimated using the HABA (4'-hydroxyazobenzene-2-carboxylic acid) method. The Thermo Scientific™ Pierce™ Biotin Quantitation Kit (Product No. 28005) contains a premix of HABA and avidin and a biotinylated protein control supplied in the convenient No-Weigh Format.

Related Thermo Scientific Products

28372	BupH™ Phosphate Buffered Saline Packs, 40 pack
66382, 66807	Slide-A-Lyzer Dialysis Cassette Kits, 10K MWCO, 3mL and 12mL, respectively
89891	Zeba Spin Desalting Columns, 7K MWCO, 5mL, 5/pkg
28005	Pierce Biotin Quantitation Kit
21911	EZ-Link Maleimide-PEG₁₁-Biotin, 25mg
21362	EZ-Link NHS-PEG₄-Biotin, 50mg
21126	Streptavidin, Horseradish Peroxidase Conjugated, 1mg
15120	Streptavidin Coated Plates, 5 plates (see catalog for a complete listing of plates)
20347	Streptavidin Agarose Resin, 2mL
20228	Pierce™ Monomeric Avidin Kit

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	Maleimide-PEG2-Biotin	Maleimide-PEG11-Biotin
Alternative names	Maleimide-PEO2-Biotin Mal-dPEG2-Biotin Maleimido-PEG2-Biotin	Maleimide-PEO11-Biotin Mal-dPEG11-Biotin Maleimido-PEG11-Biotin
Molecular formula	C ₂₃ H ₃₅ N ₅ O ₇ S	C ₄₁ H ₇₁ N ₅ O ₁₆ S
Molecular weight	525.62	922.09
Spacer arm length	29.1 angstroms	59.1 angstroms
Mass added to target	525.23 daltons	921.70 daltons
Form	White to off-white solid	White to off-white solid
Solubility	at least 25mg/mL in water or buffer	best to make DMF or DMSO stock
Storage conditions	4°C with desiccant	
Reactive groups	Maleimide, reacts with sulfhydryls at pH 6.5-7.5	
SMILES structure of Maleimide-PEG2-Biotin: O=C1NC2C(C(CCCCC(NCCOCCOCCNC(CCN3C(C=CC3=O)=O)=O)=O)SC2)N1		